THE BRITISH VETERINARY JOURNAL

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Vol. 105 **June, 1949** **No. 6**

EDITORIAL

THE ROYAL COLLEGE OF VETERINARY SURGEONS

THE passing of the Veterinary Surgeons Act, 1948, has been followed by the first change provided for in the Act, viz. the formation of a new Council of the Royal College of Veterinary Surgeons. The reconstituted Council consists of: (1) members appointed by the Privy Council, the various Universities mentioned in the Act and the affiliated Veterinary Colleges; (2) members elected by the veterinary profession; and (3) members representing the Republic of Ireland, one nominated by the Ministry of Agriculture of the Republic and four elected members. A list of the members is given elsewhere in this issue of THE BRITISH VETERINARY JOURNAL.

At its first meeting the Council elected as its President for 1949-50 T. Dalling, M.A., M.R.C.V.S., F.R.S.E., Chief Veterinary Officer of the Ministry of Agriculture and Fisheries. The newly elected Council will have a busy time during the next few years, for the various items of the Act will be brought into being and careful consideration will have to be given to the operating of the several changes provided for in the Act. New bye-laws, etc., have to be thought out and discussed, and there are many important points to which the Council will have to give its attention. We extend our congratulations and good wishes to all its members and we will follow their work with much interest.

It is to be hoped that the Universities concerned with veterinary education will very soon play their part in the training of veterinary surgeons. Some have already commenced on that work, others are about to undertake veterinary education: the remainder, we hope, will soon make the necessary arrangements with the respective Veterinary Colleges whereby they play their part in the training of veterinary surgeons of the future. The closer association of Universities with veterinary education has been discussed for some considerable time: it is now well on the way to be accomplished and we look forward with much interest to the completion and results of the arrangements. British veterinarians

stand very high to-day in the estimation of the veterinary profession throughout the world. It is a joy to visit other countries and to receive veterinary visitors from abroad and to learn how much veterinary surgeons from our own country are respected. The Veterinary Schools and the Royal College of Veterinary Surgeons have carried out their functions and duties in an able manner, and all credit is due to them. The part to be played by the Universities should assist not only in maintaining that high standard but in broadening the outlook and general experience of veterinary surgeons of the future. The linking of veterinary science with other sciences and the closer association of veterinary students with men and women interested in and studying subjects quite apart from veterinary science should improve the general outlook of our young veterinary people and should tend to increase their interest in a greater variety of subjects than possibly veterinarians in the past have had the opportunity to do. No matter, however, what form veterinary education may take in the future, it must be the duty of the Royal College to ensure that the high standard of the veterinary profession in this country is maintained. Most countries are very conscious of that standard and will, undoubtedly, watch with care and interest the improvements that should follow the passing of the Veterinary Surgeons Act, 1948.

GENERAL ARTICLES

STUDIES ON THE BIOCHEMISTRY OF PASTURE PLANTS—No. 3

The effects of the inclusion of Bracken (Pteris aquilina) in the diet of rats, and the problem of bracken poisoning in farm animals

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A REVIEW of bracken control[1] and bracken poisoning in cattle[2] have recently appeared. It is not proposed, therefore, to refer in detail to the extensive literature concerning bracken, except to point out that Davies[3] has estimated that in Wales alone there are some 152,000 acres of densely covered bracken in the 1,710,000 acres of rough grazing. Cattle are not the only farm animals known to be susceptible. " Bracken staggers " in horses is well known amongst hill-farming communities; we were only able to discover one reference[4] of the harmful effect on sheep reputed to be due to eating the plant.

The experimental production of bracken poisoning has been successfully demonstrated, both in cattle by Stockman[5, 6] and Shearer,[7] and in horses by Hadwen and Bruce.[8] Since we desire to draw special attention to this aspect of the " bracken problem," it is pertinent to summarise, at this stage, the mode of production, clinical signs, and post-mortem lesions resulting from so-called bracken poisoning.

(1) *Bracken poisoning in Cattle*

Stockman,[9] in his annual report to the Board of Agriculture, notes that serious outbreaks were exclusively reported in late summer and autumn after eating the green plant. Craig and Davies,[10] however, present circumstantial evidence that withered bracken used as bedding may have caused deaths in calves in winter. It is clear that before bracken poisoning can occur in bovines, substantial quantities must be ingested continuously over a period of 2-4 months. Gleeson[11] and Langley[12] both draw attention to the fact that rhizomes of ploughed-up bracken areas caused " bracken poisoning " when eaten by stock.

Boddie[13] makes the interesting statement that many experienced agriculturalists are firmly of the opinion that they can avoid losses among their hill cattle by removing them to low ground, free from bracken, at three-weekly intervals. He suggests that the heavy mortality of cattle from bracken poisoning, which occurred in many parts of Scotland in 1946, might be due to the requirements of the Hill Cattle Subsidy Scheme (1946), which stated that cattle must be on the hill continuously for 16 weeks.

Fletcher[4] distinguishes two types of bracken poisoning in cattle met with in practice, viz. :—

(a) The enteric type, which is by far the commoner in cattle, shows symptoms of depression, loss of appetite, high temperature (107 deg. F.), weak pulse,

enteritis with blood clots in the foul dung, and pallid conjunctivæ. Bleeding from the mucous membrane of the eyes, nostrils, vagina and anus occurs.

(b) The laryngitic type shows swelling in the throat region, roaring and difficulty in breathing, increased respirations and a high temperature.

Prognosis is grave in cattle, as most cases die within a few days of the appearance of symptoms.

At post'mortem, there are widespread, multiple, diffuse, subcutaneous and intramuscular hæmorrhages throughout the body. (See Stockman[6] and Shearer[7] for a detailed description.) Examination of the blood has failed to bring to light any significant abnormality, at least in the particular components tested. Similarly, the concensus of opinion is that bacterial infection does not play the primary rôle in the causation of the disease.

(2) *Bracken poisoning in Horses*

The condition known as " bracken staggers " appears when horses eat hay or corn contaminated with bracken; it also occurs in bracken areas during a hard winter, when food is scarce, and the plant is being used as bedding. Usually the first symptoms are unsteady gait, loss of equilibrium and general unthriftiness. The appetite is good, but the animal is inclined to constipation. As the disease progresses, there is marked inco-ordination, nervous spasms and severe intoxication. In marked contrast to the bovine condition, the temperature remains normal throughout. No particular abnormality is detected in the blood and no bacteria found. On replacing the incriminated bracken-contaminated fodder with a good ration recovery is usual, again in contrast to the position of cattle.

The pathological findings, according to Hadwen and Bruce,[8] show the brain and cord markedly congested, the pyloric end of the stomach membrane shows bleeding from eroded areas; the general condition is one of emaciation. No bacteria were isolated from the tissues which could in any way be incriminated as being responsible for the condition.

These authors attempted to extract a " toxic fraction " from the bracken with water, such extracts being administered by mouth to horses on a hay diet. No symptoms developed, from which it was concluded that whatever the toxic principle in bracken might be, it either was not extracted with water or was not active when administered in this form.

The results of this investigation showed clearly that bracken poisoning in horses can be experimentally produced by the inclusion of about 20 per cent of bracken in the hay, severe symptoms appearing within three weeks and death soon after.

(3) *Aetiology*

The present position can be summarised as follows :—

(i) *Pteris aquilina,* both the green plant, withered, or the rhizomes, if eaten in appreciable amounts, can cause a disease which may be fatal to cattle and horses.

PLATE I

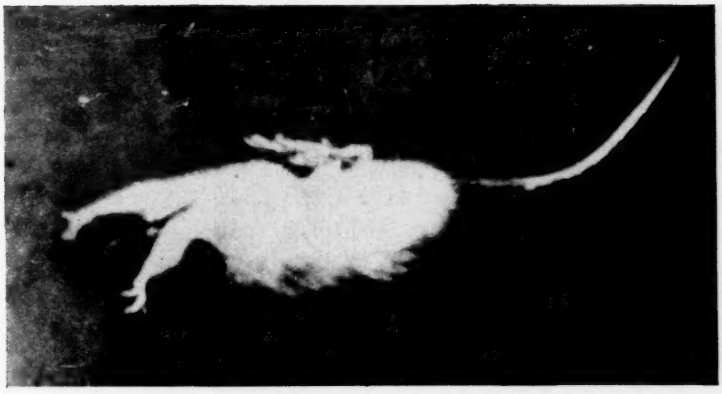
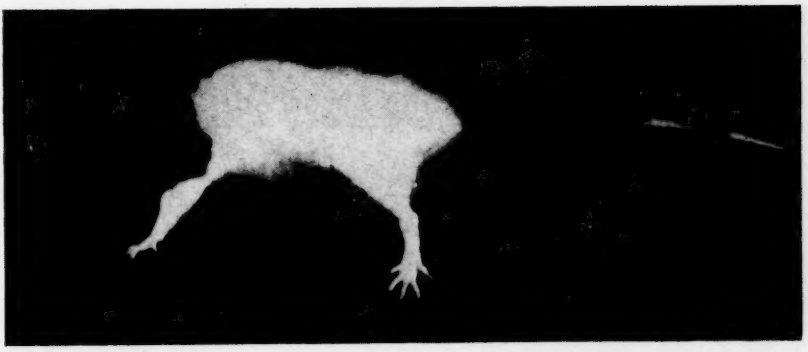


FIG. 2
Rat showing the typical nervous spasm produced by feeding the bracken 1 diet.



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(ii) The " toxic principle " is cumulative in its effect, and requires a certain time to establish its full action, after which severe illness may begin in an explosive manner.

(iii) Repeated attempts to reveal the presence of poisonous alkaloids, phytotoxins, saponins or glycosides by the usual methods have been unsuccessful, at least in such a quantity as could conceivably be responsible for disease.

(iv) Shearer[7] incriminates the " tannin " fraction of the plant, which, as Boddie[13] points out, is at its highest concentration at the period of the year when cases of bracken poisoning are most numerous. This suggestion was not followed up with any experimental evidence to support it.

Up to this time, concentration of effort was devoted to the search for a poisonous principle as such, coupled with a superimposed bacterial infection—at least in the bovine bracken poisoning.

In 1946, Weswig, Freed and Haag,[14] in a short letter, reported that on being called to investigate an outbreak of bracken poisoning, they tried the effect of feeding small laboratory animals on a diet which included the plant. Using rats, they reported the production of a diseased condition on a bracken diet (unspecified) which simulated vitamin B_1 deficiency; the animals responded to vitamin B_1 therapy. Numerous attempts in the past have been made to reproduce the condition of " bracken poisoning " in laboratory animals, especially rabbits, but apparently without success. It is obvious, however, that should this be possible, a research attack upon the mechanism of the poisoning could be carried out. Information so gained could be immediately applied towards solving the hitherto elusive problem of the causative agent in the so-called " bracken poisoning " of farm animals.

We wish to describe in this communication experiments to this end.*

Experimental

(1) *Preparation of the bracken*

Feeding experiments were performed with two samples: bracken 1 was collected in July, whilst the plant was at its full growth but still green; bracken 2 collected in October after the plant had withered, *in situ*, and was completely brown. In both cases, the leaves were carefully selected, discarding the stem entirely, so as to include the minimum amount of fibre, dried in a current of hot air at 80-100 deg. C. and then milled to powder. (In our experience with rats, a fine degree of milling is necessary to produce quick deficiency symptoms.)

Table I records the crude chemical composition of these two bracken samples, together with that of bracken at different stages of growth (data from Shearer[7]).

As can be seen, the decreasing " crude protein " and increasing values of the " crude fibre " with the ageing of the plant conforms to the usual trend. A noteworthy point is the relatively high " tannin " content at the period of maximum growth, the peak falling abruptly as the plant is allowed to wither

* A summary of this work has already appeared: W. Charles Evans and E. T. Rees Evans, Proceedings of the Biochemical Society, Jan. 22, 1949.

TABLE I

The chemical composition of bracken (Pteris aquilina) *throughout the growing season, expressed as percentage of the dry matter.*

Month	Crude Protein	Crude Fibre	Ash	Fat	Tannins	Stage of growth of the plant
May ...	21	16	8	2	2	Fronds just appearing
June ...	14	26	5	1.4	2.8	Fronds opened out
July ...	9	31	6	1.2	7	Plant at full growth
September ...	7	33	5.6	2	6	Plant starting to wither
October ...	5	40	5	2	0.4	Plant completely brown
Bracken 1 (leaf only) ...	15	18	5.5	3	8.5	Plant at full growth but still green (July)
Bracken 2 (leaf only) ...	7	30	4.1	1	0.9	Plant completely withered and brown (Oct.)

naturally. (Tannin estimations were carried out by the Loewenthal method as given by Rosenthaler.[15] This method of estimating the tannins is open to objection, as we are well aware, and steps are being taken to employ the more specific hide powder method, in collaboration with the British Leather Manufacturers' Research Association.)

(2) *Feeding experiments using rats*

The basal diet used in the rat experiments was as follows:—

	Parts	
B.D.H. Light White Soluble Casein ...	20	
Sucrose	60	
Arachis Oil	15	
B.D.H. "Dunn" Salt Mixture ...	5	
Dried Yeast	10	(vitamin B_1 content 60 μg. per gram.)

Groups of young Wistar hooded rats were selected immediately after weaning, so as to form fairly uniform batches, and the sexes segregated. The control groups were fed the above diet. The experimental groups received the same diet, with the addition, intimately mixed with it, of the bracken powders 1 or 2, in the ratio of 60 per cent basal diet to 40 per cent bracken powder. All the animals received a regular supplement, by pipette, of "radiostoleum" (6 drops per week) and a-tocopherol acetate (2 drops per week), and had access to water *ad libitum*. The rats were kept usually two per cage (dimensions 14 by 9 by 9 inches), the cages being provided with a wire-meshed false bottom so that the fæces fell through, out of reach of the animals. Weights were regularly recorded.

As expected the control groups gave normal growth curves. The group on bracken 1 diet, however, started losing weight after a few days; within a period of one month the rats were emaciated and showed nervous symptoms characteristic of aneurin deficiency. Unless treated, these animals succumbed. It is

noteworthy that the food consumption decreased appreciably on the bracken 1 diet, which is characteristic of avitaminosis B_1. Another batch of animals on this diet were observed very carefully and, a few days before death was anticipated, were injected subcutaneously with a normal saline solution

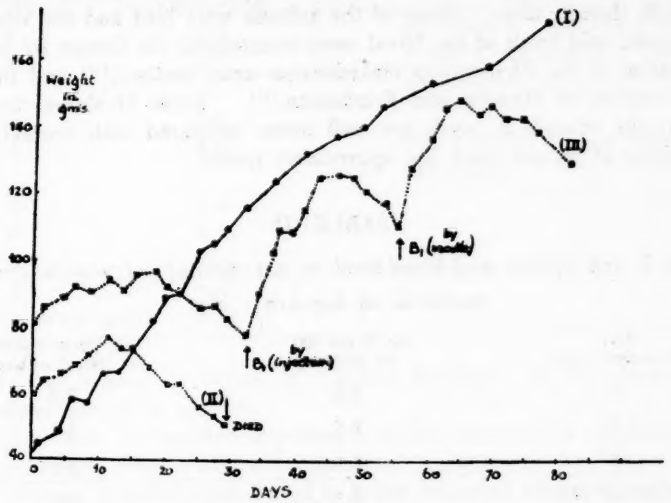


FIG. 1

Weight curves of rats on different diets.

1. Shows normal growth on the basal diet, which contains more than 600 micro-grams of vitamin B_1 per 100 gms. of the food.
2. Shows the result on the bracken 1 diet; the animals die within one month.
3. Shows the effect of the administration of small amounts of B_1 (arrows) either by injection, or by mouth, to the rats suffering from a deficiency condition produced on the bracken 1 diet. As seen, the response is dramatic.

containing 0.1 mg. of pure aneurin. A dramatic improvement resulted in every case, as shown by the liveliness of the animals, disappearance of deficiency symptoms, and prompt live-weight increase. On continued feeding of the bracken 1 diet, this batch lost weight and condition, and on being dosed with aneurin solution (0.1 mgm.) per os, by pipette, showed the dramatic response as when treated by injection. Fig. 1 shows the averaged growth curves of the various groups. Fig. 2 shows a rat with the polyneuritic symptoms produced on the bracken 1 diet.

It is known that rats grow at a normal rate on a diet containing 80-100 μg. of vitamin B_1 per 100 gms. of food. Bracken 1 diet contained, by virtue of the yeast added to it, more than five times this amount, yet with these diets avitaminosis B_1 develops.

Surprisingly, if bracken 2 was used in the rat feeding experiments, the animals thrived and did not develop the typical symptoms described above. We conclude from these experiments that bracken is more toxic to rats at certain stages of growth, some samples even being quite innocuous. Bracken taken from the same rick as sample 2 was also fed to a horse in a proportion

approaching 50 per cent of the ration, chaffed with hay; the horse showed no ill-effects within one month.

Proof that the symptoms shown by the rats on the bracken 1 diet were in fact due to a vitamin B_1 deficiency, did not rest on the prompt response to vitamin B_1 therapy alone. Some of the animals were bled and the vitamin B_1 and pyruvic acid levels of the blood were ascertained, the former by Sinclair's modification of the Phycomyces blakesleeanus assay method,[16] and the latter by the method of Haugen and Friedmann.[17] Table II shows clearly the position; the vitamin B_1 levels are well down compared with normal values, whilst those of pyruvic acid are appreciably raised.

TABLE II

Vitamin B_1 and pyruvic acid blood levels of rats showing polyneuritic symptoms, produced on bracken 1 diet.

Rat (Bracken 1 diet)		µg. B_1 per 100 ml. of blood		mg. pyruvic acid per 100 ml. of blood
1	.....	3.9	.....	2.8
2	.....	2.5	.....	3.1
3	.....	1.5	.....	3.9
Normal rats	.....	8.5	.....	0.9
(basal diet)	.....	(7-10)	.....	

(3) *The vitamin B_1 blood levels of cattle recovering from bracken poisoning*

Through the kindness of our colleague, Mr. L. E. Hughes, M.R.C.V.S., D.V.S.M., the Veterinary Investigation Officer at Aberystwyth, an opportunity arose for some preliminary observations on the vitamin B_1 blood status of some cattle which were recovering from bracken poisoning in the field. The treatment consisted of a change of diet. From this herd, six animals had succumbed a week previous to the time the blood samples were taken. All the animals had been out since May, 1948, on the rough hill land, which contained areas abundantly covered with bracken. The typical symptoms developed in August, 1948, and were followed by the quick death of half a dozen of them. The animals listed in Table III eventually recovered; the two marked with an asterisk were the only ones which showed elevated temperatures at the time of bleeding.

As can be seen, the vitamin B_1 blood level of a few of these animals is still well below the normal level, thus supporting the thesis that in bracken poisoning of cattle there is also a vitamin B_1 deficiency. Confirmation and amplification of these findings as regards vitamin B_1 and pyruvic acid levels of the blood in cases of bracken poisoning will be sought at the earliest opportunity. If substantiated, there would be reasonable grounds for predicting that vitamin B_1 therapy should be effective in its treatment.

TABLE III

Vitamin B_1 blood levels of cattle recovering from "bracken poisoning." Llanidloes, August, 1948.

Animal	μg. B_1 per 100 ml. of blood	Remarks
1 ...	4.8 ...	All animals appeared dull.
2 ...	5.0 ...	4 and 8 had shown a high temperature (up to 107° F.).
3 ...	5·5	
4* ...	4.0* ...	Six animals died a week previously.
5 ...	6.0	
6 ...	6.2	
7 ...	6.5	
8* ...	3.8*	
Normal values	8-12	

(4) *The mechanism of action of "active bracken" in the production of avitaminosis B_1 in the rat.*

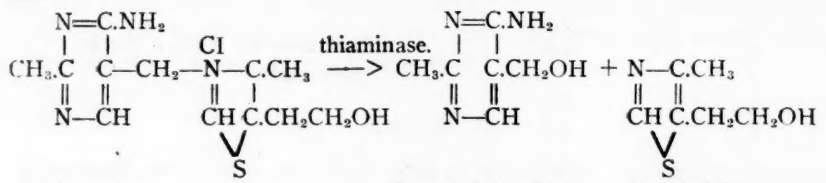
Apart from the applied importance of the observations described in this communication, there is also a fundamental problem raised: "How does the active bracken powder, when added to a diet otherwise adequate in its vitamin B_1 content, cause symptoms of deficiency of this vitamin to develop in the rat?"

It is a well known fact in animal nutrition that many of the B vitamins are not only present in natural foodstuffs which constitute the diet, but are synthesised by the intestinal bacteria and may become available to the animal from this source. From modern biochemical views on the ways that vitamins can be inhibited from functioning normally in the intact animal, we can enumerate five possible modes of action, viz.:

(a) Failure of intestinal absorption.
(b) The destruction or inactivation of the vitamin by certain enzymes, substances or bacteria.
(c) The inhibition or change of the normal bacterial flora producing the vitamin by antagonists or the absence of an essential metabolite.
(d) The displacement of the vitamin from the tissues by analogues.
(e) Exposure of the organism to special stresses, such as infections, and a bad balance of the major components of the diet, especially at such critical periods as pregnancy.

In view of our results with the inactive bracken 2, which is much lower in crude protein and almost double the crude fibre content of the active bracken 1, we are inclined to dismiss possibility (e) forthwith. That bracken either contains a substance antagonistic to vitamin B_1, an enzyme which inactivates it, some substance combining with or in some way changing it chemically, seems to us the most likely explanation of the phenomenon, i.e., mode of action (b) above.

The discovery by Green, Carlson and Evans[18] that chastek paralysis, a disease of foxes caused by feeding raw carp could be prevented or cured by aneurin, calls to mind a possibly analogous state of affairs which may have some bearing on the problem under discussion. The mechanism in this case has been considerably clarified by Krampitz and Woolley,[19] who found that an enzyme (" thiaminase ") occurred in several aquatic animals which inactivated vitamin B_1 by splitting the molecule into its pyrimidine and thiazole components, thus :



Aneurin — 2-methyl-4-amino -5-hydroxymethyl pyrimidine — 4-methyl-5-hydroxytheyl thiazole.

These two parts of the aneurin molecule possess no vitamin activity for mammals, although certain micro-organisms, e.g. *Phycomyces blakesleeanus* can resynthesise the complete vitamin from these components.

Another interesting example of the inactivation of aneurin, in this case by certain foodstuffs and oil seeds, has recently been reported by Bhagvat and Devi.[20] Utilising certain Indian plant products, e.g. ragi (*Eleusine coracana*), rice polishings, bajra (*Pennisetum typhoideum*), green gram (*Phaseolus radiatus*), mustard seed, cotton seed and linseed, they found that all contained a factor which rapidly inactivated aneurin in vitro. This anti-aneurin factor was found by these authors to be non-enzymic in character and therefore presumably quite different from the thiaminase of fish tissue. The mode of inactivation of vitamin B_1 by the plant extracts still remains unknown.

5. *Incubation of bracken leaf and rhizome extracts with aneurin in vitro.*

Bracken leaf 1 and 2 and bracken rhizomes collected from a recently ploughed field (January, 1949), heavily infested with the plant, were all extracted separately with water containing a few drops of chloroform, using " chatteris quartz " to macerate the tissues. For comparison, some perennial rye grass was treated similarly. All the extracts were filtered clear and 10 ml. of each, equivalent to 0.5 gm. plant material, added to 10 ml. portions of 0.1 M. acetate buffer of pH 5.6 in separate flasks. To each flask, 100 μg. of vitamin B_1 were added, together with a few drops of toluene as a preservative, each flask stoppered and then incubated overnight at 37° C. A control flask, containing vitamin B_1 in acetate buffer alone was also included. After incubation the vitamin in each flask was estimated, both by the thiochrome method according to the procedure of Harris and Wang[21] and the Phycomyces assay method. The results obtained are shown in Table IV.

As can be seen, bracken 1 extract, which was active in producing avitaminosis B_1 in rats, contains a factor extractable with water, which inactivates

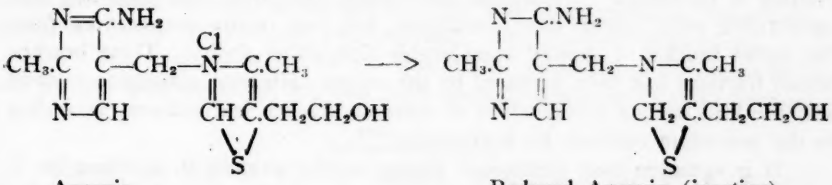
TABLE IV

Incubation of vitamin B¹ (5 μg. per ml.) with various plant extracts, and the subsequent activities as demonstrated by the thiochrome and Phycomyces methods of estimation.

Extract	% destruction of B_1 Thiochrome method	Phycomyces method
Bracken 1	90	Nil
Bracken 2	5	Nil
Bracken rhizome	90	Nil
Perennial rye grass	Nil	Nil

vitamin B_1 in vitro, as measured by the chemical method. The rhizome extract is also active in this respect. Neither bracken 2 extract, which, as will be recalled, was inactive in the rat experiments nor perennial rye grass possessed this property. The products of incubation of the vitamin were, in all cases, equally active by the Phycomyces assay method. It is already known that this fungus does not necessarily require the intact aneurin molecule for growth in a synthetic medium, but can utilise the thiazole and pyrimidine components just as well. We regard such experiments as tentative pointers that " active " bracken contains a substance, relatively thermostable, which probably splits the aneurin molecule.

There are, however, several other ways in which aneurin could be changed chemically so as to be inactive for mammals, for instance the bracken may contain reducing agents which act on the vitamin, producing the reduced form which is known to be devoid of vitamin activity, cf. Lipmann,[22] thus:



Aneurin — Reduced Aneurin (inactive)

Moreover, this reduced form is non-autoxidisable. A decision has to await chemical isolation.

A word of warning is necessary in the interpretation of results obtained by the thiochrome method for the estimation of vitamin B_1 in plant materials. Grob[23] has already drawn attention to the lack of agreement between the Phycomyces and thiochrome methods when applied to spinach or cabbage extracts, the latter method giving low values.

6. *The incubation of substances, mainly of plant origin, with aneurin in vitro.*

The test substance (10 mg.) was dissolved in acetate buffer 0.1 M. pH 5.6 (10 ml.), vitamin B_1 added (50 μg.), together with a drop of toluene, stoppered, and incubated overnight at 37° C. Vitamin B_1 was then estimated in each sample, using the thiochrome method. The intensities of the blue fluorescence in ultra-violet light were compared and are recorded in Table V.

TABLE V

Incubation of vitamin B_1 with different substances which occur in plant tissues.

Substance	Degree of intensity of the thiochrome reaction remaining after incubation, control as 100%
Control	100
Ascorbic acid	10
Gallic acid	10
Protocatechuic acid	10
Pyrogallol	10
Tannic acid (oak galls)	80
Tea tannin	90
Phloroglucinol	70
Catechol	70
Glutathione	100
Bracken rhizome extract	5
" " (boiled)	90
Bracken 1 tannin fraction (Copper carbonate method)	10
Bracken 1 tannin fraction (Lead acetate method)	5

As can be seen, the presence of auto-oxidisable substances like ascorbic acid, gallic acid, pyrogallol and protocatechuic acid causes considerable loss. Neither a tea-tannin* preparation nor tannic acid from oak galls had any appreciable effect under these conditions, but two tannin preparations from the active bracken 1 showed considerable destructive ability. These bracken tannin fractions had been prepared by the copper carbonate absorption method and the lead acetate precipitation of aqueous bracken leaf extracts, according to the procedure outlined by Rosenthaler.[15]

It is apparent that continuous testing on the vitamin B_1 deficient rat is essential before deciding whether the vitamin has in fact been inactivated for mammals by any particular procedure, and these experiments are in progress.

Discussion

It is now well established that the various B vitamins are not dietary essentials for ruminants, as they are produced by the symbiotic action of micro-organisms in the rumen. Conditions are much less favourable for microbic synthesis in mono-gastric herbivora. The view is suggested in this communication that bracken poisoning of farm animals is primarily an avitaminosis B_1, produced by the action of an inactivating principle in bracken which destroys the vitamin in the fodder, and also that part contributed by the micro-flora of the alimentary tract. The fact that the condition is easier to reproduce in horses than in cattle is in conformity with this view. The symptoms described in

* We are grateful to Dr. A. E. Bradfield, of the East Malling Research Station, for supplying this preparation.

" bracken staggers " of horses are also, in the main, those that would be expected from vitamin B_1 deficiency. In cattle, the position is somewhat more complex; evidence has been presented of vitamin B_1 blood levels definitely subnormal in animals recovering from so-called bracken poisoning. We are at a loss, at present, to explain the increased temperatures which invariably accompany the disease.

The position will be clarified when an opportunity is offered to study the condition in the light of the new knowledge now gained. Field trials will then decide whether the ætiology of this pasture-grazing animal disease is the same as that produced artificially in rats. If such is the case, vitamin B_1 therapy should quickly restore the animals to normal health, provided that other complications have not set in.

Summary

1. The production, in rats, of a disease simulating vitamin B_1 deficiency, by the inclusion of bracken in a diet containing an adequate supply of the vitamin, has been confirmed. Indications of differences in the toxicity of bracken according to the stage of growth has been obtained.

2. Vitamin B_1 blood levels have been shown to be subnormal, whilst the pyruvic acid content of the blood is high in these animals. Vitamin B_1 therapy, either by injection or by mouth, is effective in curing the condition.

3. Cattle just recovering from bracken poisoning showed low vitamin B_1 blood levels.

4. Aqueous extracts of bracken leaf and rhizome inactivated vitamin B_1 when incubated in vitro, as estimated by the thiochrome method; the activity, as shown by the Phycomyces assay method, was, however, unimpaired.

5. The mode of action of the bracken in producing avitaminosis B_1 in the rat has been discussed, together with bracken poisoning of farm livestock.

Acknowledgments

Thanks are due to Mr. R. Phillips, M.Sc., and Mr. L. E. Hughes, M.R.C.V.S., D.V.S.M., for their help. We are grateful to the Director of the Welsh Plant Breeding Station for granting us field facilities, and to Mr. Harry Jones for his help with the farm animals. Mr. L. Bebb very kindly placed his bracken rick at our disposal. Finally, we thank Miss Savage and her assistant for the care of the laboratory animals.

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AN INDIRECT METHOD OF DETERMINING THE SYSTOLIC BLOOD PRESSURE IN DOGS BY THE USE OF GRANT'S CAPSULE

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and
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THE problem of determining the systolic and diastolic blood pressures in the dog without puncture of the skin, and yet with simplicity and accuracy, is one which remains unsolved. In the laboratory the difficulty is surmounted by such procedures as exteriorisation of the carotid artery, which are clearly inapplicable under the conditions obtaining in clinical work. An attempt was therefore made to evolve a technique of estimating the blood pressure by an indirect method which, at the same time, would give results with reasonable accuracy and consistency.

The literature contains accounts of a variety of methods which have been employed in an attempt to solve the problem, and several of these have been tried by the present writers. A particularly careful trial was given to methods based on those employed in man, in which a Riva-Rocci sphygmomanometer cuff is used to obliterate the artery. In this method the systolic and diastolic pressures are determined by auscultation and palpation respectively. Although this method appeared to offer the most promising approach, it was found to be impracticable in the majority of animals, although both fore and hind limbs were used. It was found that the vasoconstriction which occurs in the peripheral

half of the femoral artery, even in only slightly chilly surroundings, was such that the pulse volume was reduced to a point where it became extremely difficult to detect it in many dogs either by palpation or auscultation. There is the additional drawback to this method that in the femoral region the hind leg of the dog has the form of an elliptical truncated cone as contrasted with the cylindrical form of the human forearm, for which the Riva-Rocci cuff was originally designed. In practise, therefore, the application of such a cuff to the femoral region of the dog results in a large proportion of the pressure being dissipated in distorting the shape of the limb to a more nearly cylindrical shape, with relatively little pressure being exerted upon the artery itself. This objection was overcome by Kolls (1920) by the use of a specially designed cuff, but without using his somewhat elaborate method of detecting the systolic and diastolic pressures, the earlier difficulty mentioned above remains.

These difficulties led to a trial of the present indirect method. Though by no means ideal, it was found to be applicable in the majority of dogs and to have many points in its favour compared with other methods.

Principle

The method was originally designed by Grant and Rothschild (1934) for the estimation of the blood pressure in the rabbit, and for a full description of the apparatus and principles involved, reference may be made to their paper. In brief, the technique consists in the application to the shaven ear of a transparent airtight pressure capsule which rests over the central artery of the ear; behind the ear and connected with the capsule by a horseshoe-shaped clip is a metal plate carrying a window in line with the transparent capsule. The surface of the capsule in contact with the ear consists of a transparent distensible membrane which, when the pressure in the capsule is raised, assumes a dome shape and presses the artery against the window. The capsule is closed with a small glass disc, through which the vessel is viewed. The artery is rendered clearly visible by transillumination of the ear from a light source. The systolic reading is represented by the pressure required to cause complete cessation of blood flow along the artery. The diastolic pressure cannot be obtained with accuracy by this method, and therefore no attempt has been made to obtain diastolic readings.

Anatomical Considerations

While the ear of the dog is not so ideally suited by the method as is that of the rabbit, for which the method was originally described, a well-defined central artery is present in the majority of dogs. The arterial supply of the ear of the dog comes from two sources:

(a) The anterior auricular artery. This is a branch of the superficial temporal artery and is the less well defined of the two ear arteries. It supplies branches to the anterior and medial aspects of the ear.

(b) The posterior auricular artery. This vessel is a branch of the external carotid and forms the main supply of the ear. Emerging from beneath the parotid gland at the base of the ear, it runs up the medial aspect

of the concha, distributing branches to the medial and posterior aspects of the ear. An anterior branch of this artery also supplies the anterior aspect of the ear and anastomoses with the anterior auricular artery.

In general, it has been found that the main trunk of the posterior auricular artery was a fairly large and distinct vessel and that the remaining vessels were comparatively small. In a proportion of dogs, however, the anterior and posterior vessels are of approximately the same calibre and appear to anastomose freely, with the result that no well-defined central artery exists. In these the satisfactory application of the method is often difficult or impossible.

Description of Apparatus

Although the capsule as originally designed for rabbits and at present marketed would probably have served equally well with the majority of dogs, it was thought desirable to make provision for the great variation in the sizes of dogs. Instead, therefore, the two arms of the clip were made so that the distance between them was adjustable. This was effected by making the clip in two parts. These were then screwed together with a washer between them, corresponding in size to the degree of separation between the two arms of the clip that is required. The optimum distance apart, of course, will be governed by the thickness of the ear of the animal on which the capsule is to be used. Two washers, 1/8th in. and 3/16th in. in thickness, were found to be adequate to meet the requirements of varying thicknesses of ears. The rest of the apparatus consists of a mercury manometer of the type often used with the Riva-Rocci cuff in man. This is connected with a pressure bottle of about 500 c.c. capacity, which, in turn, is connected with the small brass tube on the capsule. An ordinary electric light bulb, partly enclosed in a shade, was used to transilluminate the ear; this in addition served to provide the necessary warmth to the ear. The pressure in the system is raised by a rubber pressure bulb carrying a valve.

Preparation of the Animal

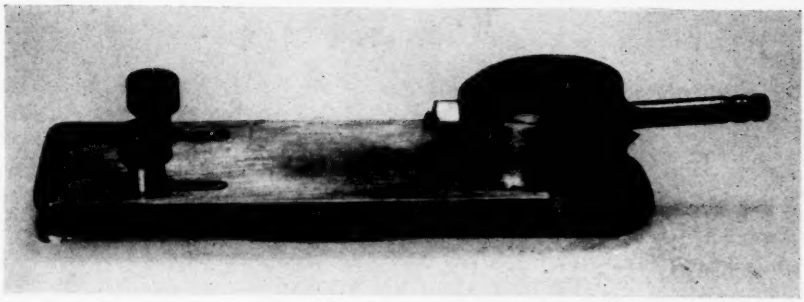
It is absolutely essential for the success of the method that the dog should not be either frightened or in any way excited in the period preceding the actual taking of the reading; it is important therefore that the animal should be handled as quietly as possible.

The ear is lathered with warm soap and water and shaved over an area about 1½ in. square, in its proximal half. The warmth of the electric light bulb is then allowed to play upon the ear, taking care that the light is not held too close. It was usually found that a hitherto somewhat restive animal, if held by an assistant in this position for a few minutes, would gradually relax under the warmth of the electric light bulb.

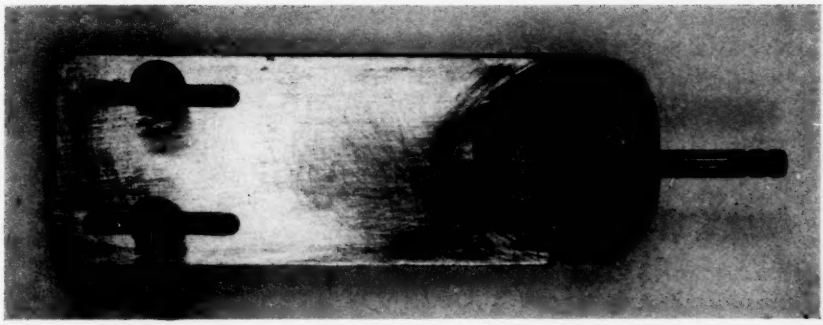
Technique

After warming for a few minutes, a little 70 per cent glycerine is smeared over the shaven part of the ear and the capsule applied. It will have been seen already that on holding the ear to the light a network of vessels presents itself. Most of these very prominent vessels are, however, veins and on appli-

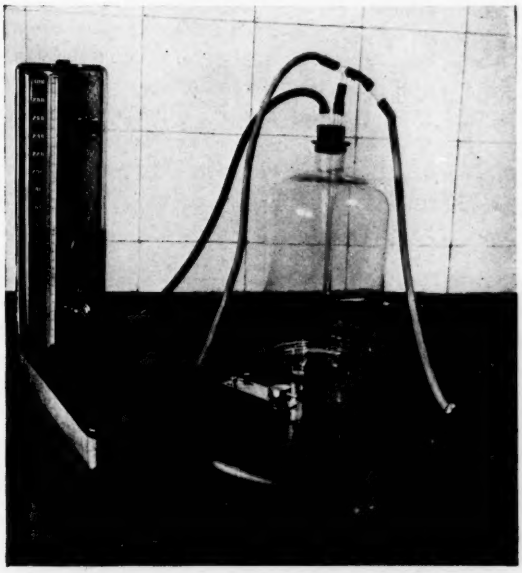
PLATE I



Grant's Capsule (side view).



Grant's Capsule (from above).



Apparatus when assembled ready for use.

Article by Platt and Vivian, page 186

cation of the capsule and raising the intracapsular pressure to 30 mm. of mercury or so, they collapse within the area covered by the capsule leaving the field comparatively avascular. The central artery should be sought approximately mid-way between the anterior and posterior borders of the ear. If the ear is not thoroughly warm there may be some little difficulty in detecting the vessel owing to vasoconstriction. On vasodilatation, however, the vessel can be seen as a well-defined structure with more sharply defined edges than is seen in the veins. If the artery is not easily detected at the outset, it is best sought for by applying the capsule and raising the capsular pressure to about 70 mm. of mercury. With glycerine on the ear, the capsule can be moved about the ear without discomfort to the animal and should be slid to and fro over the normal position of the artery and any vessel which looks like the artery. At the above pressure the veins will collapse where the centre of the capsule rests over them. Also, since 70 mm. of mercury approximates to the diastolic blood pressure in the ear, when the artery is encountered, the distension of the capsule to this pressure ensures that the pulsation in the vessel will show its greatest amplitude. Though in many dogs the artery can be found readily, in some a few minutes' careful search may be required before the pulsation of the vessel can be detected, and this appears often to be facilitated by varying the capsular pressure between 70 and 100 mm. of mercury occasionally. For an accurate reading it is essential that the vessel wall should be relaxed as much as possible; partial vasoconstriction gives falsely low readings. In this connection it will be noted in practice that the earlier readings in an animal are frequently much lower than those obtained a few minutes later, when more complete vasodilatation has occurred. This, in the writers' experience, was an early source of error, and animals from which readings of less than 90 mm. of mercury were obtained (without obvious clinical cause) should be subjected to further readings a few minutes later without removing the capsule in the meantime. It will often be found that with further warming, more complete vasodilatation has occurred and that the occlusion now takes place at a considerably higher pressure. The relaxation in arterial tone often occurs very abruptly.

The end point is determined as follows: With an intracapsular pressure rather below that of the diastolic blood pressure it has already been noted that arterial pulsation is very distinct. If the capsular pressure is now raised so that it is greater than the diastolic pressure but less than the systolic pressure, the resistance offered by the centre of the capsule to the wall of the vessel against which it is pressed is such that the blood can only be forced past the obstruction during systole. As a result, the blood is forced along the artery in a series of spurts, each spurt coinciding with cardiac systole. If the pressure is now raised still further, to a point just above that of the systolic pressure, blood flow along the artery will be completely arrested. The pressure required just to obstruct all flow along the vessel represents the systolic blood pressure. This point is best found by raising the pressure to about 10 mm. of mercury above obliteration point and allowing the pressure to drop gradually, and noting the

pressure at which the first spurts of blood again begin to course along the artery. Three successive readings are obtained, all within 5 mm. of mercury of each other, to ensure that the blood pressure is fairly constant. The method then adopted by the writers has been to accept as the final reading the figure represented by the nearest 5 mm. of mercury above these three readings. For example, if three readings are recorded as 104, 103, 101, then 105 is taken as the final result. This method is adopted, firstly, because to record readings to the nearest mm. of mercury is to credit the whole procedure with an accuracy which it does not possess. Also, this method will tend to eliminate to some extent the slight discrepancies between the actual systolic pressure point and the pressure present in the capsule at the time when it can be said with certainty that spurts of blood are again traversing the vessel.

Fallacies of the Method

False readings as a result of incomplete vasodilatation have already been mentioned. Alarm or fear on the part of the dog with consequent rapid fluctuation in the blood pressure may also cause misleading results, but if several readings are taken in succession, wide variation between them is usually noted. In such circumstances it is best to abandon proceedings for the time being.

Limitations

Although the method can be applied in the majority of dogs, for one reason or another it has failed in about 25 per cent. The latter consist mainly of dogs with thick ears, such as spaniels, and dogs with large amounts of melanin in the hair and skin. A combination of these factors usually renders transillumination of the ear impossible. In a small proportion of cases, usually in association with one or both of the above contraindications, it has been impossible to locate either the central artery of the ear or any other suitable vessel. The biggest single drawback to the application of the method in the dog as a part of the routine clinical examination is the amount of time involved. In those animals in which the skin of the ear is not pigmented and the central artery is well defined, a reading can be obtained fairly rapidly, but in a larger proportion of dogs, especially those of a nervous disposition, this is not the case, and on this account the employment of the method as a part of the routine clinical examination cannot be entirely recommended. The method, however, might have considerable value in experimental work in which dogs with suitable ears can be selected. The validity of the results obtained by this procedure may be questioned on the grounds that the ear has not been sympathectomised as is always done in the experimental rabbit. Clearly, the use of the method for the ordinary clinical work, for which it has been employed by the writers, precludes any such surgical measure. However, it was felt that if successive readings are taken to ensure that the blood pressure is at a fairly constant level, many of the objections to this omission will be obviated.

Readings

During the time that the method has been employed by the writers as part of the routine clinical examination of the dog, readings have been obtained from 20 animals in which, as far as could be ascertained, the blood pressure lay within normal limits. A summary of these readings follows. The figures refer to systolic blood pressure only.

Range: 95-115 mm. of mercury.

Mean: 100.5 mm. of mercury.

Standard deviation: ±7.8 mm. of mercury.

Correlations: There was no significant correlation between age and blood pressure ($r=+.265$), nor between weight and blood pressure ($r=+.303$). The coefficient of partial correlation between age and blood pressure, but eliminating the effect of weight, was also insignificant ($r=+.22$).

Acknowledgments

We wish to record our indebtedness to Professor G. Payling-Wright for his help and criticism in the writing of this paper, and to Mr. R. H. Knight for the photographs.

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STUDIES ON EQUINE PURPURA HÆMORRHAGICA
Article No. 4—Hæmatology

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THE three previous papers in this series have been concerned with (1) the earlier literature on equine pupura hæmorrhagica (Biggers and Ingram, 1948), (2) the clinical observations on the cases we have investigated (Bennett and King, 1948), and (3) the morbid anatomy and histopathology of these cases (King, 1949). In this paper the hæmatological observations which we have made will be described in detail since they help to correlate the morbid anatomy, histopathology and the biochemical findings (the latter to be reported in the next paper of this series). In general, the results confirm the earlier work of Mielke (1913), Wittman and Contis (1924) and Jennings and Highet (1947).

The investigations undertaken fall into two major sections, and will be presented in turn:—

(a) Studies on the red blood cells,

(b) Cytological studies.

Also a few observations have been made on the blood clotting mechanism in this disease.

(a) Studies on the Red Blood Cells

From each of the cases a series of blood samples was taken (see Bennett and King, 1948); the samples, with certain exceptions, were withdrawn once every 24 hours, as far as possible at the same time each morning.

The following determinations were made :—

(a) *Red blood cell counts*

The cells were enumerated in a Thoma counting chamber using a dilution of 1 in 200.

(b) *Packed cell volume*

The blood was placed in a Wintrobe tube and centrifuged for six minutes.

(c) *Blood viscosity*

This was determined in a modified Ostwald viscometer. The results are expressed relative to water (water—1).

(d) *Hæmoglobin determinations*

The total oxygen capacity of each of the first twelve samples was determined by the manometric method of Van Slyke and Neill (1924). From these the hæmoglobin concentrations were calculated, assuming that 1 gm. of hæmoglobin combines with 1.34 cc. of oxygen. Simultaneous determinations of the hæmoglobin concentrations were made using the Haldane hæmoglobinometer. When the hæmoglobin concentrations were calculated taking the 100% standard as being equivalent to 14.6 gm. hæmoglobin per 100 cc. of blood, an appreciable discrepancy was found between these calculated values and the results of the Van Slyke determinations. Accordingly, the standard tube was recalibrated from the data already obtained. This new standard was used for all subsequent blood samples; Van Slyke determinations were discontinued. These results show the necessity for frequent recalibration of the hæmoglobinometer standard.

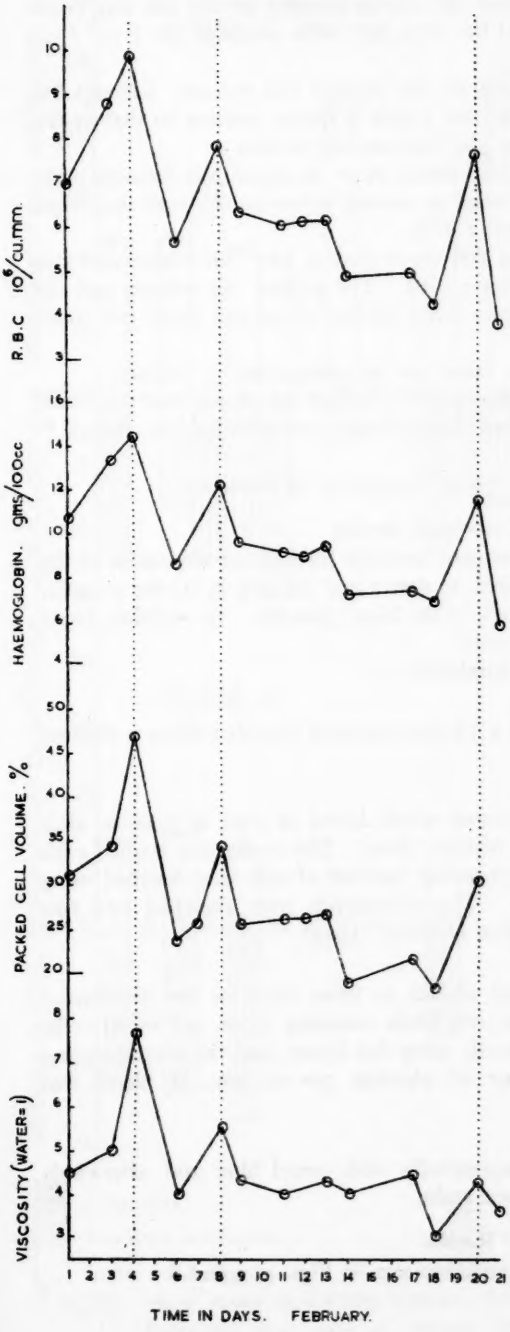
With the exception of the points mentioned above all the techniques were carried out according to the methods described by Holman (1944) and Stewart and Holman (1940). The packed cell volumes were determined immediately after collection of the blood samples; the other estimations were done as soon as possible afterwards.

Results

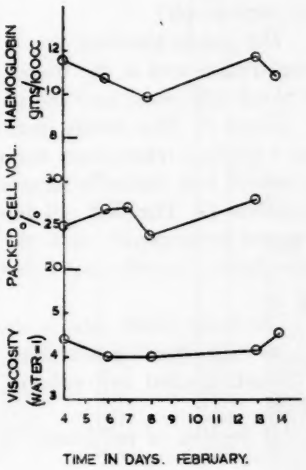
These are shown graphically in Figs. 1, 2 and 3.

Horse C. The first graph (Fig. 1), illustrating the daily red blood cell counts during the course of the illness, shows two marked features. First, a steady decline of the red blood cell count from a first day value of 7.0×10^6 / cu. mm. to a 21st day value of 3.7×10^6 / cu. mm.; and secondly, three sharp rises in the general trend of the graph where there is a well defined increase in the red blood cell count. The first rise, with a maximum on the 4th day, reaches a value of 9.9×10^6 / cu. mm. (normal range $5.7 — 8.8 \times 10^6$ / cu. mm., Stewart and Holman, 1940). Thus, during the first rise there was an increase of approximately 3.0×10^6 / cu. mm. over the original value. The second and

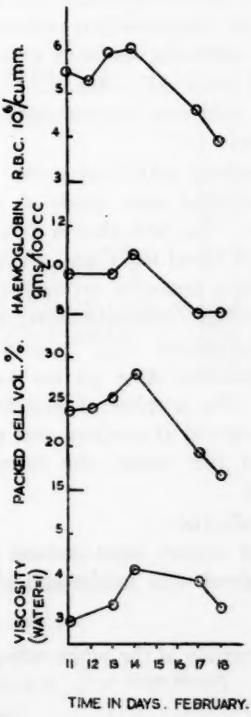
HORSE C. (Fig.i)



HORSE F. (Fig.ii)



HORSE G. (Fig.iii)



third periods of increased red blood cell counts occured on the 8th day (with a rise of 2.5 x 10^6 / cu. mm.) and the 20th day (with a rise of 3.0 x 10^6 / cu. mm. respectively).

The graphs showing the course of the packed cell volume, hæmoglobin concentration and of the blood viscosity follow a similar pattern to that of the red blood cell count, and the same two features can be seen.

Horse F. The results from this animal show no significant features other than a gradual return from sub-normal to normal values on the 10th day when the animal had clinically recovered (Fig. 2).

Horse G. The first red blood cell count gave a very low value, and this decreased progressively until the horse died. The packed cell volume and the hæmoglobin concentration followed a trend similar to the red blood cell count (Fig. 3).

The main points seen in these cases can be summarized as follows:—

(1) A progressive anæmia—shown by a gradual decrease in the red blood cell count, packed cell volume and hæmoglobin concentration, in Horses C and G.

(2) Periods of red blood cell count fluctuations in Horse C.

(b) Cytological Studies

Observations were made at frequent intervals throughout the course of the disease, in each of the animals studied, to detect any changes in (1) the granular leucocytes, (2) the agranulocytes, and (3) the blood platelets. In addition, blood smears were examined for reticulocytes.

The following methods were employed:—

(a) *Total white cell count*

The cells were enumerated in a Thoma counting chamber using a dilution of 1 in 10.

(b) *Differential white cell count*

Blood films were made of citrated whole blood as soon as possible after collection. This was always done within 1 hour. The smear was stained with Leishman's blood stain, and an appropriate number of cells was classified using the Schilling meander technique. The neutrophils were classified into two groups, mature and immature, after Holman (1944).

(c) *Platelet counts*

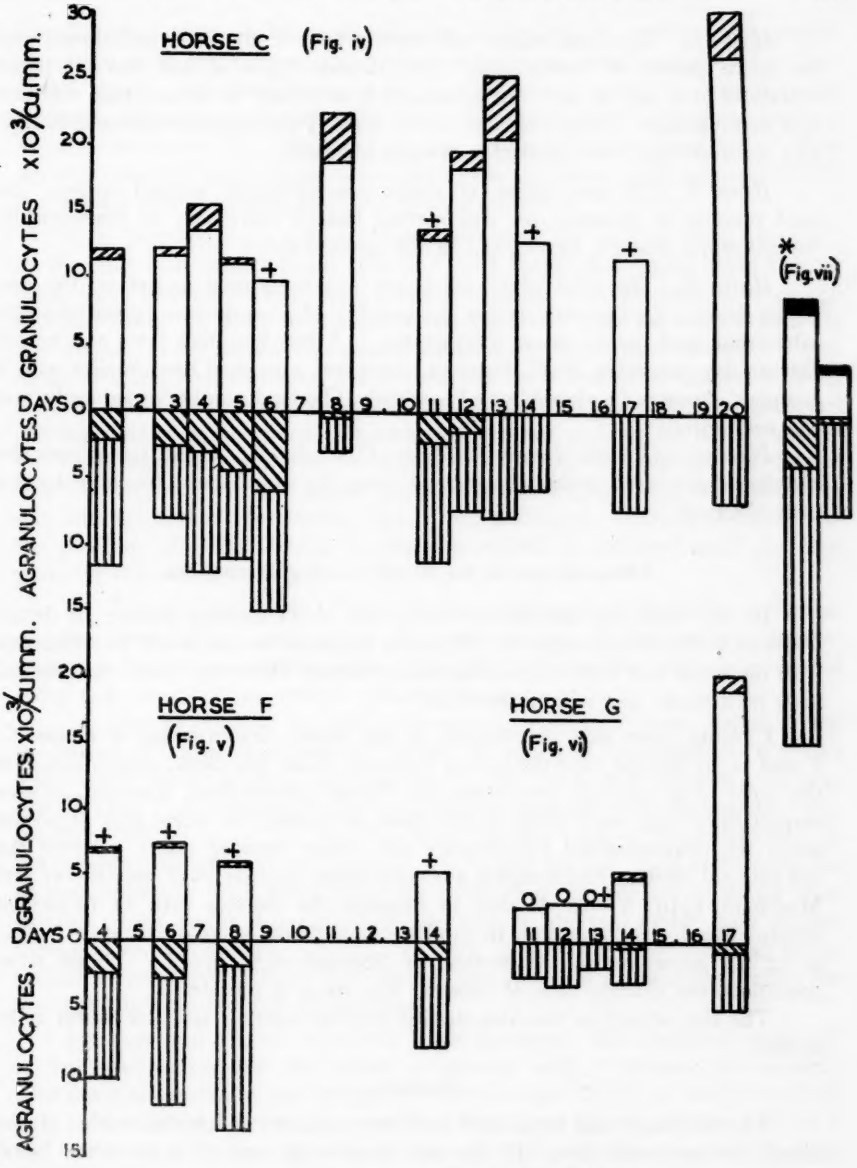
These were done on the same smears as those used for the differential counts. The number of platelets seen while counting 1,000 red blood cells, in fields selected at random, was noted; using this figure, and the corresponding red blood cell count, the number of platelets per cu. mm. of blood was calculated.

(d) *Reticulocytes*

Blood smears were stained supravitally with cresyl blue and afterwards counterstained with Leishman's blood stain.

Results

The results of the white cell counts are seen in Figs. 4, 5 and 6.



* NOTE. Fig.vii shows MAXIMUM NORMAL RANGES. (1 σ from means)
Calculated from data of Holman (1944)

Horse C. The total white cell count was well above normal throughout the entire period of observation. The various types of cell showed rapid variations from day to day. In general, it is seen that the leucocytosis was due to a neutrophilia. There was also a very marked eosinopoenia (aneosinophilia). The agranulocytes were present in normal numbers.

Horse F. The total white cell count was within the normal range. The total number of granulocytes was normal though there was an eosinopœnia. No abnormal changes were noted in the agranulocytes.

Horse G. The total white cell count was subnormal except on the day before death. In the early stages the number of granulocytes appeared to be subnormal, and there was an eosinopœnia. A few basophils were also noted. On the day preceding death, however, there was a marked neutrophilia with a complete absence of eosinophils and basophils. The total number of agranulocytes was subnormal.

Platelets and Reticulocytes in Horses C, F and G. In all three cases the platelet counts were within the normal range for the horse. No reticulocytes were observed.

Observations on the Blood Clotting Mechanism

In our cases we intended to study the blood clotting picture in detail. Unfortunately certain technical difficulties impeded the proposed investigations with the result that little information was obtained. However, a few observations have been made, and will be described.

Clotting times were determined on the freshly drawn blood of Horses C, F and G, by the test tube method of Lee and White (see Beck, 1938). Times of the order of 40 minutes were obtained. These observations, however, became suspect since they were made in the open air during the worst days of winter when the environmental temperature was below freezing point. Since the biochemical findings had revealed a normal blood calcium level (see Harvey and Marchant, 1949) it was decided to measure the clotting time of recalcified citrated blood. This was done in capillary glass tubes (internal diameter, 1 mm.) at 37°C., according to the method of Szabuniewicz (1932). Under these conditions the clotting time of horse C was about 6 minutes.

The clot retraction was also studied in these animals and was found to be normal.

Discussion

The discussion will be divided into three sections which deal with: (I) the blood clotting mechanism, (II) the red blood cells, and (III) the white blood cells.

I. *The blood clotting mechanism.* The results of the platelet counts in these cases supports the view of Grieg (quoted by Hutyra *et al.*, 1946), and the finding of Jennings and Highet (1947), that the disease is not associated with a thrombocytopœnia. A mistranslation of the original paper of Wittman and

Contis (1924) seems to be responsible for the reports of a thrombocytopœnia, associated with the disease, in recent text-books of veterinary medicine (e.g. Udall, 1943). Wittman and Contis, in fact, record either a normal or an increased thrombocyte count. Recently, Aggeler *et al.* (1946) have found a distinct correlation between blood platelet counts and clot retraction. Thus our observation of normal clot retraction in the animals studied supports further the finding that the blood platelet counts are normal.

It is, perhaps, not too hazardous to compare the clotting time, at 37°C., on the recalcified citrated blood of horse C with the normal equine blood clotting times published by Stewart and Holman (1940). These workers employed capillary tubes and made their observations at 37°C. Thus the two methods are not unlike. They found the normal range to be 4.5—12.0 minutes. Our figure of 6 minutes, therefore, lies towards the lower end of this range. Bouchet and Bouchet (1927) and Bellocq and Louf (1943) have described an increased clotting time in equine purpura hæmorrhagica; but, judging by the account of the symptomatology and morbid anatomy, it is doubtful whether the former were dealing with a case of equine purpura hæmorrhagica. The inference from our findings is that the clotting time is not prolonged. Further discussion of the problem of blood clotting in this disease will be deferred until the final paper of this series.

II. *The red blood cells.* From our own hæmatological studies, and those of other workers, two main points of interest emerge, (1) the presence in some cases of a progressive anæmia, and (2) the relatively rapid fluctuations in the red blood cell count. These will be discussed in turn.

(1) The progressive anæmia.—Previous investigators have described the occurrence of a progressive anæmia during the course of the condition (Wittman and Contis, 1924; Jennings and Highet, 1947). Also, it is interesting to note that in 1843 Field mentioned the association of anæmia with the disease. The results of Mielke (1913), on the other hand, of four cases of *Petechialfieber,* do not support this observation; however, his figures for red blood cell counts are so extraordinarily high throughout his paper that we are led to doubt their validity. Our results demonstrate clearly that, in the two severe cases studied (horses C and G), anæmia occured. In both there was a progressive fall in the number of red blood cells per unit volume, together with an associated fall in the hæmoglobin concentration of the blood.

Jennings and Highet, however, when discussing their findings, make the further observation that the attack *commences* with a pronounced anæmia. This is not supported by the findings of Wittman and Contis, in which six of the nine cases examined showed, on the first day, a red blood cell count of 7.0 x 10^6 / cu. mm. or more, and only one case showed an initial count of less than 6.5. The findings reported above on horse C, in which the initial red blood cell count was 7.0, provide further evidence that the condition does not always commence with a marked anæmia.

The exact cause of the anæmia in this disease is difficult to explain. If the disease was simply hæmorrhagic in nature it could easily be classed as a post-

hæmorrhagic anæmia resulting from a compensatory haemodilution. However, the post-mortem evidence reported by King (1949) is not consistent with this view. He has suggested that in this condition hæmorrhage is subsidiary in importance compared to œdema formation. In fact, in both horse C and G, where anæmic changes were extreme, only a little hæmorrhage was found post mortem; indeed, in horse C it was difficult to find any hæmorrhage at all. A possible explanation of this anæmia is that it is similar to the *anæmia of infection* of man. This type of anæmia has been analysed recently, from a clinical and an experimental standpoint, by Cartwright *et al.* (1946 a, b). They suggest that it is due to an interruption in the synthesis of the hæmoglobin molecule in the body. An analysis on similar lines in equine purpura hæmorrhagica is suggested. This would involve studies on (a) the histology of the bone marrow and (b) the metabolism of iron in affected animals. We feel that, until such studies are done, interpretation of the reports of icterus (Hutyra *et al.*, 1946; Belocq and Louf, 1943) and of a high icteric index (Jennings and Highet, 1947) is difficult. It is desirable, in connection with this suggestion, that investigations be carried out on the properties of the red blood cells after such infectious diseases as strangles and influenza. To our knowledge, *anæmia of infection* is not recognised as a distinct entity in veterinary medicine. If it occurs it may explain those cases of equine purpura hæmorrhagica where anæmia is already present when the condition is diagnosed.

(2) Fluctuations in the red blood cell counts.—The most interesting case which we have studied is horse C. This case progressed slowly with recurrent exacerbation of symptoms. The graph of the day-to-day red blood cell counts shows three rises, with maxima on the 4th, 8th and 20th days; these were associated with similar rises in the packed cell volume and hæmoglobin concentrations. (See figure i. Continuous dotted lines are drawn through each of these three maxima). The rises were relatively rapid in onset and may be considered to be periods of hæmoconcentration*. (Felger [1948] has reported fluctuations in the red blood cell count of the normal horse which are attributed either to differences in the time of day at which the blood sampling was carried out, or to excitation of the animal during venepuncture. During our work care was taken to avoid these disturbances as far as possible. Also, as the fluctuations which we found in horse C are larger than those found by this worker we consider that they are significant.) Similar high red blood cell counts have been reported in the literature. Case V of Wittman and Contis (1924) showed one count of 9.1×10^6 / cu. mm., and case IX showed an initial count of 9.0 and a count of 10.0 three days later shortly before death. Mielke's cases also show some remarkably high counts, even up to 13.5, but, for the reasons given above, his figures cannot be relied on. Hitherto, the significance of the occurrence of high red cell counts in equine purpura hæmorrhagica has not been discussed in the literature.

In horse C, these three periods of hæmoconcentration coincided exactly

* In this paper, hæmoconcentration means a relatively rapid increase in the red blood cell count, due to a loss of plasma volume, i.e., a sudden increase in the cell-plasma ratio.

with the three clinical crises which occurred during the course of the disease (see Bennett and King, 1948). The last of these crises, it is true, was not distinguished by any adverse fluctuation in the œdema; but the first crisis was marked by the temporary attainment of a maximum distribution of the œdema, and the second was marked by the resumption of its formation. We consider that these periods of hæmoconcentration, especially when coincident with signs of œdema formation, indicate that plasma constituents were being lost into the tissues. This interpretation supports the hypothesis, derived from the morbid anatomical and histological findings (King, 1949), that damage to the capillary endothelium, resulting in abnormal permeability to plasma colloids, may be one of the factors involved in the pathogenesis of equine purpura hæmorrhagica.

To sum up, examination of the data derived from the study of the red blood cells shows that when analysing the pathogenesis of this disease, two types of case must be considered; (1) where death occurs in association with hæmoconcentration, such as case IX of Wittman and Contis, and (2) the more common variety where death occurs in association with anæmia. This will be discussed more fully in the final paper of this series (Biggers *et al.*, 1949).

III. *The white blood cells.* The cytological studies made on the blood of the affected animals showed a marked leucocytosis in horses C and G. This has also been found by Mielke (1913), Wittman and Contis (1924) and Jennings and Highet (1947). Like Jennings and Highet we have found this leucocytosis to be due mainly to a neutrophilia. Our observations also agree with their's in that an eosinopœnia was found. Although our results, shown in fig. 4, indicate the distribution of monocytes and lymphocytes which we have found, comment has been withheld since great difficulty was experienced in classifying many of the cells observed, and consequently complete reliance cannot be placed on the figures obtained.

Summary

(1) Studies on the hæmatology of three cases of equine purpura hæmorrhagica have been described.

(2) The disease is a non-thrombocytopœnic purpura.

(3) The blood clotting mechanism is probably normal.

(4.) The disease is commonly associated with an anæmia; this should be further studied.

(5.) Rapid rises in the red blood cell counts are considered to be periods of hæmoconcentration.

(6) The occurrence of periods of hæmoconcentration is thought to indicate that plasma constituents are being lost into the tissues.

(7) Death may occur in association with either hæmoconcentration or anæmia.

(8) The condition is associated with a neutrophilia and an eosinopœnia.

Acknowledgements

We are indebted to Messrs. P. M. Bennett and A. S. King and to Miss G. Halley for their invaluable assistance in carrying out some of the observations described; to Mrs. J. D. Biggers for help in preparation of the manuscript; and to Mrs. A. S. King for drawing the figures.

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CAUSES OF STERILITY IN BULLS IN SOUTHERN ENGLAND

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(Continued)

18. (AB 33, Testicular Degeneration)

History.—Guernsey, 2 years old. Purchased at the age of 1 year and 5 months. Served 24 cows in 7 months without success. Daily ration comprised dairy cake 2 lb., oats 3 lb., and hay 17 lb. Received no exercise. Previous service 17 days before examination.

Behaviour at service.—" 2," cow not in heat. Stroked a few times inside prepuce before penis came out. Penile rigidity subnormal, resulting in prolonged vulvar titilation before insertion. This was a persistent feature.

Clinical examination.—Testicles three-fifths the normal size, soft and flabby in texture and often kept in tucked-up position. Badly " dished " back.

Advice.—Retesting after 2 weeks.

Subsequent observations.—Re-examination confirmed previous findings. Condition being advanced, slaughter was recommended.

Autopsy.—Testicles small, as stated before. Sections showed 5 per cent tubules in a hypoplastic state (no development beyond spermatogonia) and 95 per cent in various stages of degeneration: desquamation of germ cells and the presence of multi-nucleated cells in tubular lumina. Spermatogenesis, with

the presence of a large number of spermatids, was evident in most tubules. Separation of sperm heads from the tails was present everywhere. A few sperm heads with tails were disintegrated and markedly eosinophilic, with or without a collar or bleb around the middle-piece.

19. (AB 35, Testicular Degeneration)

History.—Guernsey, 2½ years old. Was purchased at the age of 2 years. Fertility rate in 6 months preceding examination was 23 per cent. Daily ration comprised beet-root pulp 4 gallons, and hay *ad lib.* Previous service 15 days before examination.

Behaviour at service.—" 1," cow not in heat. Disliked artificial vagina and did not ejaculate into it until 10 days later and thereafter.

Clinical examination.—Normal.

Advice.—Nil, bull was going for slaughter.

Autopsy.—Sections showed from 8 per cent (right) to 12 per cent (left) tubules in hypoplastic state (only one or two layers of spermatogonia). Remaining tubules were in various stages of degeneration: desquamation and accumulation of spermatogenic cells in tubular lamina, formation of multi-nucleated cells and vacuolisation of cell layers comprising spermatocytes. The number of spermatocytes and spermatids in most tubules was reduced considerably. Wherever spermatogenesis had progressed to its final stage, sperm numbers were extremely small.

20. (AB 4, Testicular Hypoplasia)

History.—Shorthorn, 3 years old, premium bull. Father had reputation for good fertility. Was first put to service when 1¾ years old. Fertility rate declined from 40 per cent in the first three-quarters to 10 per cent in the fourth quarter of the first year of service. Total number of cows and heifers served in the first year was 98. All cows served during 2 months preceding examination failed to conceive. Daily ration comprised dairy concentrates 3 lb., mangolds or kale 20 lb., and meadow hay *ad lib.* Received no exercise. Previous service 4 weeks before examination.

Behaviour at service.—" 2," cow not in heat.

Clinical examination.—Testicles and epididymes three-fourths the normal size.

Advice.—Retesting after 2 weeks.

Subsequent observations.—Nil, slaughtered without intimation.

21. (AB 18, Testicular Hypoplasia)

History.—Shorthorn, 1½ years old. Was first put to service when 1 year old. Regularly served 25 cows and heifers for months but none became pregnant. Daily ration comprised dairy concentrates 5 lb., mangolds 20 lb., and hay *ad lib.* Was kept in a small dingy loose-box and never received any exercise. Previous service 15 days before examination.

Behaviour at service.—" 1," cow in heat. Erection, insertion and ejaculation were instantaneous. Refractory state after each ejaculation passed into one of readiness to serve again in a few seconds before dismounting.

Clinical examination.—Testicles three-fifths the normal size, left slightly smaller than right. Epididymes three-fourths the normal dimensions. Scrotum often seen tucked-up.

Advice.—Retesting after 2 weeks.

Subsequent observations.—Hypoplasia established and slaughter recommended.

Autopsy.—Testicles and epididymes small as stated above. Sections showed all seminiferous tubules in a hypoplastic state. Most of them were lined by one or two layers of spermatogonia only and a few showed progress towards spermatogenesis up to the stage of spermatocytes, spermatids and rarely spermatozoa. In the latter case desquamation of spermatogenic cells was almost always present.

22. (AB 25, Testicular Hypoplasia)

History.—Shorthorn, 2 years old, well-bred bull. Was first put to service when 1 year old. Regularly served 20 cows and heifers for 9 months but none became pregnant. Daily ration comprised oats 3 lb., grass-meal 8 lb., and a sufficient quantity of meadow hay. Ration was supplemented with high quality proteins for a few months but without any improvement. Received exercise for 30 minutes daily. Previous service 3 months before examination.

Behaviour at service.—" 1," a quiet bull was used in place of a cow. Refractory period after ejaculation similar to AB 18.

Clinical examination.—Testicles one-half the normal size. Epididymis one-half the normal size on right and practically unpalpable on left side. Eight-month-old brother of the bull on the same farm was examined and found to be a monorchid.

Advice.—Retesting after 2 weeks.

Subsequent observations.—Hypoplasia established and slaughter recommended.

Autopsy.—Testicles and right epididymis small as stated before. Left epididymis about one-fourth the normal size (it was quite difficult to cut a piece of reasonable size from the tail for sectioning). Sections were similar to those of AB 18. Four-fifths (right) to three-fifths (left) of seminiferous tubules were hypoplastic and the remainder showed a partial progress towards spermatogenesis. Left testicle of the monorchid brother was of normal size and texture, with progress in spermatogenesis up to spermatocytes in all tubules. Right testicle, found in the upper part of the inguinal canal, was soft and one-half the size of the left testicle. Its tubules were lined by a single row of spermatogonia only.

23. (AB 36, Testicular Hypoplasia)

History.—Guernsey, 2 years old. Was first put to service when 1 year old. Right testicle descended at 15 months. Served 30 heifers during first year of service unsuccessfully. Semen test carried out some time ago showed oligospermia with nearly all sperm dead. Daily ration comprised dairy concentrates 8 lb., potatoes 25 lb., and sufficient seeds hay, with grazing whenever possible.

Behaviour at service.—" 1," cow not in heat. Otherwise similar to AB 18.

Clinical examination.—Left testicle turgid, right one-fourth normal size and very soft (fœtal consistency). Epididymis one-half the normal size.

Advice.—Slaughter recommended, but has not yet been carried out.

24. (AB 7, Balanitis)

History.—Ayrshire, 2 years old. Was purchased at the age of 1½ years. Served 9 cows unsuccessfully. Each showed vaginal discharge 3 days after service; this soon turned yellow and fœtid. Daily ration comprised dairy concentrates 4 lb., mangolds or kale 15 lb., and sufficient hay. Grazed regularly. Previous service 7 days before examination.

Behaviour at service.—" 2," cow not in heat.

Clinical examination.—Normal. Cows with discharge showed vaginal catarrh or purulent vaginitis with or without nodule formation on vulva and the posterior part of vaginal wall.

Advice.—Insertion into prepuce of " pentaflav " bull cones twice a week for 3 weeks, preceded by a douch of preputial cavity with a mild antiseptic each time.

Subsequent observations.—Examination of prepuce and its washings after treatment showed freedom from balanitis. Another examination after 3 months' rest (because affected cows were still under treatment) confirmed a healthy state of prepuce. Bull was allowed to be used for breeding purposes, and is working normally since.

25. (AB 19, Balanitis)

History.—Guernsey, 3 years old, well-bred bull. Was first put to service when 9 months old. Fertility rate in the year preceding examination was 50 per cent; previous records not available. Was used to serve cows in the open, and always showed sluggishness at service. Daily ration comprised 3 lb. of oats, hay and outside grazing in summer, and oats 4 lb., kale 12 lb., silage 12 lb., and hay in winter. Previous service 11 days before examination.

Behaviour at service.—" 7," cow not in heat. Displayed no interest in cow. Change of cow or walking her in front of him failed to elicit any sign of sex arousal.

Clinical examination.—Preputial mucosa nodular and congested.

Advice.—Retesting when a cow in heat was available.

Subsequent observations.—Heard nothing from owner; efforts to contact him failed.

26. (AB 29, Balanitis)

History.—Shorthorn, 7 years old. Was standing idle for 6 months owing to inflammation of prepuce. There was trichomonad infection in the herd. Fertility rate before the period of idleness was 80 per cent. Daily ration comprised a mixture of cake, oats, beans and wheat 2 lb., mangolds and sugar-beet 20 lb., and enough hay.

Behaviour at service.—" 1," cow not in heat. Penis bled after first ejaculation.

Clinical examination.—Nodular inflammation of preputial lining with considerable reddening of glans penis.

Advice.—Retesting after 20 days.

Subsequent observations.—Balanitis, of trichomonad origin nearly established. Owing to owner's proposal to dispose of the bull no treatment recommended.

27. (AB 2, Paunchiness)

History.—Jersey, 7 years old. Became progressively slow at service and, at times, failed to serve altogether during the year preceding examination (fertility rate prior to that was normal). Of 3 cows served during that year, 2 aborted at 7-8 months (of these the one served first was a new acquisition and had already aborted once on her way to the herd), while the third bore her calf normally. Both cows showed vaginal discharge for several days after abortion. Daily ration comprised dairy concentrates 4 lb. and hay 40 lb. or more. Received very little exercise. Previous service 3 months before examination.

Behaviour at service.—" 7," cow in heat. Penis erected partially and there was desire to mount the cow (evidence by posture), but he did not jump.

Clinical examination.—Abdomen abnormally large and pendulous, about 10 inches from ground. Preputial orifice inflamed and sensitive. Masturbated occasionally.

Advice.—Daily ration of concentrates to be increased to 8 lb. and hay reduced to 25 lb., with 45 minutes' vigorous exercise daily. Treatment for preputial catarrh was not prescribed to observe the pathogenicity of *Absidia corymbifera* harbouring his prepuce.

Subsequent observations.—After 3 months' paunchiness and preputial catarrh disappeared, masturbation stopped and behaviour improved to " 3." He started serving cows satisfactorily. Owner was advised to continue instructions regarding feeding and management.

28. (AB 5, Paunchiness)

History.—Guernsey, $2\frac{3}{4}$ years old, well-bred bull. Was first put to service when $1\frac{1}{2}$ years old. Fertility rate was normal, but was slow at service. Daily ration comprised dairy concentrates 3 lb., greens 20 lb. and hay 40 lb. or more. Received no exercise. Previous service 15 days before examination.

Behaviour at service.—" 4," cow not in heat. Erection was immediately followed by an attempt to mount the cow, but there was hesitation for a considerable time before he actually mounted her. This was a persistent feature.

Clinical examination.—Very large abdomen.

Advice.—Daily ration of concentrates to be increased to 10 lb. and hay reduced to 17 lb., with one hour's brisk exercise daily.

Subsequent observations.—After $2\frac{1}{2}$ months abdomen became well tucked-up, behaviour improved to " 2 " and he commenced breeding satisfactorily. Owner was advised to continue changes in management. Another visit about a year later showed that he was working normally.

29. (AB 13, Paunchiness)

History.—Ayrshire, $2\frac{1}{4}$ years old, well-bred bull. Fertility rate was normal, but was slow at service. Daily ration comprised concentrates 5 lb., mangolds

25 lb. and hay 35 lb. or more. Received no exercise. Previous service 6 days before examination.

Behaviour at service.—"4"-"5," cow not in heat. Penile rigidity subnormal. Stroked inside prepuce 2-4 times before penis came out every time.

Clinical examination.—Abdomen very large and moderately pendulous.

Advice.—Daily ration of concentrates to be increased to 7 lb. and mangolds and hay reduced to 15 lb. each, with 45 minutes' daily exercise.

Subsequent observations.—Re-examined 6 months later. Instructions had not been carried out. His condition was unchanged. Cowman wanted to get rid of the bull and there appeared no hope of correcting management.

30. (AB 3, Faulty Erection)

History.—Jersey, 3 years old. Was first put to service when 1 year old. Entangled himself with a 70 ft. long rope used for tethering and fell down in a ditch about 5 months before examination. Owner noticed no serious injury. A week later tried to serve but failed owing to improper erection: penis did not erect to its full length. A course of 3,000 units of PMS showed no beneficial effect. Daily ration comprised concentrates 5 lb. (in breeding season only) and enough hay. Was tethered outside for grazing and exercise regularly. Previous service 3 months before examination.

Behaviour at service.—"2," cow in heat. Penis came out of prepuce only for 5-6 inches and showed subnormal rigidity. Could not reach cow's vagina but there was no difficulty in collection by artificial vagina. On subsequent jumps penis did not come out at all, although he mounted the cow several times.

Clinical examination.—Proximal part of perineum on right side sensitive to palpation. Stiffness of lumbo-sacral region and lameness of right hind leg. On each step the leg was lifted with a certain amount of jerk combined with extra flexion at hock, and put down on the ground with hesitation.

Advice.—Regular grooming and massage of perineum and lumbar region with ammonia liniment twice daily for 4 weeks. Daily ration to consist of concentrates 8 lb., silage 12 lb. and sufficient hay and greens. Complete sexual rest.

Subsequent observations.—After treatment showed nearly normal erection at first jump, but reverted to the original state at second and third jumps at 15 minutes' interval. Owner had decided to send him for slaughter; further advice was not contemplated.

Autopsy.—Marked fibrosis with adhesions of skin to underlying structures over first sacral and second and third lumbar vertebræ. Parts of semimembranosus covering ischio-cavernosus and bulbo-cavernosus muscles lacerated and œdematous. Deeper examination impossible due to the regulations of the Ministry of Food. Genital organs normal on macro- and micro-scopical examinations.

31. (AB 24, Faulty Erection)

History.—Guernsey, $1\frac{3}{4}$ years old. Was first put to service at the age of

11 months, when he failed to reach cow's vagina owing to imperfect erection. Was standing idle since then. Owner noticed reduction in size of testicles in a few months' time. Daily ration comprised concentrates 4 lb. and regular grazing on fields growing rye grass (60 per cent), cocksfoot (30 per cent) and white clover (10 per cent).

Behaviour at service.—" 1," cow not in heat. Erection similar to that of AB 3. Masturbated occasionally.

Advice.—A course of PMS. Daily ration of concentrates to be increased to 8 lb.

Clinical examination.—Testicles three-fifths the normal size and soft. Stiffness of sacro-lumbar region and lameness of right hind leg like AB 3.

Subsequent observations.—Instructions not followed and bull sent for slaughter without intimation.

32. (AB 41, Faulty Erection)

History.—Fresian, 5 years old, well-bred bull. Was first put to service when 1 year old. Fertility rate normal until 4 months before examination, when he jumped over a fence and hurt himself. Excepting a superficial long wound along the prepuce, no other injury was noticed. After the wound had healed, he attempted but failed to serve owing to imperfect erection. Daily ration comprised concentrates 10 lb., mangolds and kale 20 lb., and sufficient hay.

Behaviour at service.—" 1," cow not in heat. Mounted but could not take penis out of prepuce for more than 3 inches.

Clinical examination.—Slight lameness of right hind leg like AB 3. Examination after casting under epidural anæsthesia revealed nothing of interest: penis could not be taken out even after tiring the retractors.

Advice.—A course of PMS.

Subsequent observations.—Treatment not yet carried out.

33. (AB 40, Unknown Disorder)

History.—Hereford, 2¼ years old. Was first put to service when 1¾ years old. Of first 6 cows served, 5 became pregnant. Of next lot of 8, whom he served more than once, none conceived. Became progressively slow at service and ultimately refused to serve altogether. A course of 3 injections (1,500 units each) of PMS did not effect improvement. Daily ration comprised concentrates 10 lb., mangolds 12 lb. and hay as much as he could eat. Received little exercise.

Behaviour at service.—" 7," cow in heat. Was completely indifferent to and disinterested in the cow.

Clinical examination.—Fat deposition around spermatic cord and in proximal part of scrotum. Well-fed beef condition.

Advice.—Daily ration to comprise of concentrates 3 lb., mangolds 8 lb. and hay 10 lb. for 1 month, with vigorous exercise.

Subsequent observations.—Still under observation.

REVIEW

TEXTBOOK OF MEAT INSPECTION by H. Thornton, B.V.Sc., D.V.H., M.R.C.V.S., pp. xi + 656, 4 colour plates and 257 other illustrations. Price 50/-. Lond, Baillière, Tindall & Cox.

GREAT Britain has never regarded meat inspection in this country very seriously although she has adopted a very different attitude in connection with those countries which export meat to Great Britain. While she has been content with a rather haphazard system of inspection at home she has forced exporting countries to comply to a code of inspection which has necessitated the establishment of efficient veterinary meat inspection services. Meat inspection in this country has been conducted, in the main, too much in a "rule of thumb" manner rather than on a scientific basis, and this attitude has been reflected in the dearth of literature and textbooks on the subject; indeed, for a great number of years, a German work was the only means of reference available to the British veterinary meat inspector. Thornton's book fills the gap.

When one first glances through the book one is struck by the fact that, although it is a first edition, it has the appearance of an established textbook which has run to more than one edition and is so mature and well balanced that one can hardly realise it is a first edition. One is impressed with the extensive and wide reading which the author must have undertaken over a number of years, with repeated checking of the script, to keep the work up to date.

In addition to the more narrow conception of "meat inspection," poultry, rabbits and fish inspection is described as also are subjects of ancillary interest such as the preservation of food, including canning, treatment and disposal of by-products and fats, all of which are of special interest to sanitary inspectors and other public health workers.

The author has wisely omitted to give reprints of various Acts and regulations applicable to meat inspection in this country which so frequently occupy much space in similar types of textbooks and which are liable to be superseded and are of purely "local" interest but he has discussed in detail some of the directions given in the codes of inspection practised in Great Britain and endeavoured to give a fair interpretation of them. His views will be shared by most experienced meat inspectors and, although some may possibly disagree with some of his conclusions, in the main there will be general agreement.

The book is well supplied with illustrations, a few of which, unfortunately, are not up to the same high standard as the script and are too small or too indefinite to be readily intelligible to those unacquainted with the subject.

Thornton is to be congratulated on producing the most concise and authoritative textbook on meat inspection so far produced in this country and the future of the book is assured. It will find a ready sale amongst veterinary students, sanitary inspectors, students studying for the diploma of the National Federation of Meat Traders Associations, and others studying the subject of food inspection, whilst it will prove a most useful book of reference for all public health workers interested in the subject of food hygiene.

THE ROYAL COLLEGE OF VETERINARY SURGEONS

CONSEQUENT upon the operating of the Veterinary Surgeons Act, 1948, the Council of the Royal College of Veterinary Surgeons has been reconstituted and held its first meeting on June 1, 1949. The Council consists of *appointed* and *elected* members. The following is its constitution:—

APPOINTED MEMBERS.—*Privy Council*: P. H. Collick, M.P., W. McNair Snadden, M.P., T. Dalling and H. W. Dawes. *Universities*: F. Blakemore (Bristol), W. I. B. Beveridge (Cambridge), F. A. E. Crew (Edinburgh), R. C. Garry (Glasgow), J. G. Wright and R. E. Glover (Liverpool) and J. B. Hunter (London). *Affiliated Veterinary Colleges*: W. M. Mitchell (Royal [Dick] Veterinary College, Edinburgh), A. A. Forsythe (Glasgow Veterinary College) and J. B. Buxton (Royal Veterinary College, London).

ELECTED MEMBERS.—R. F. Montgomerie, T. M. Mitchell, L. Guy Anderson, L. P. Pugh, J. T. Edwards, G. N. Gould, J. R. Barker, H. Sumner, G. H. Wooldridge, G. F. Boddie, G. P. Male, J. W. Procter, T. J. Bosworth, W. R. Wooldridge. H. W. Steele-Bodger, J. W. Emslie, J. D. Peele, J. F. D. Tutt, J. Shane-Jones and W. R. McKinna.

In addition, the representatives of the Republic of Ireland are: T. G. Brown (nominated by the Ministry of Agriculture of the Republic), P. F. Dolan, W. Kearney and P. E. Mullaney (elected members). There is still one vacancy for an elected member from the Republic.

The Council unanimously elected T. Dalling, M.A., M.R.C.V.S., F.R.S.E., Chief Veterinary Officer, Ministry of Agriculture and Fisheries, as President for the year 1949-50, and he presided at the first meeting. The following were elected as Vice-Presidents: H. W. Dawes (Senior Vice-President), J. G. Wright, J. F. D. Tutt, J. W. Procter and J. Shane-Jones (Junior Vice-Presidents). Professor J. B. Buxton was re-appointed Treasurer and Mr. W. G. Oates was re-elected Secretary and Registrar.

Under the new Act a Disciplinary Committee is now specially appointed, one of whose members must be a Privy Council nominee. H. W. Dawes was appointed in this capacity. The Disciplinary Committee consists of H. W. Dawes (Chairman), R. E. Glover, J. B. Buxton, A. A. Forsythe, L. Guy Anderson, G. F. Boddie, T. M. Mitchell, L. P. Pugh and H. Sumner.

On the day following the first meeting of the Council a luncheon was held at which were present the members of the new Council and those who had constituted the previous Council, together with a number of guests who are closely associated with veterinary science and agriculture, including the Rt. Hon. Tom Williams, Minister of Agriculture and Fisheries. Short speeches were made by the President, the Minister and Mr. H. W. Dawes.

Publishers' Notices

THE BRITISH VETERINARY JOURNAL, with which is incorporated THE VETERINARY JOURNAL, is published monthly, and copy for advertisements should be in the hands of the publishers—Baillière, Tindall and Cox—not later than the 20th of the preceding month, if proof is required. Tel.: Temple Bar 3386.

Letters for the Journal, literary contributions, reports, notices, books for review, exchanges, new instruments or materials, and all matter for publication (except advertisements) should be addressed to the Editor.

Annual Subscription, 30s. ($6.50 U.S.A. currency, post free.)